

**AMENDMENTS TO THE SPECIFICATION**

**Please amend the specification as follows:**

**Page 1, after the Title and before line 1, insert**

**STATEMENT OF GOVERNMENT INTEREST**

This invention was made with support pursuant to National Institutes of Health Grant No. RO1 AI29471. The government may have certain rights in this invention.

**CROSS-REFERENCE TO RELATED APPLICATIONS**

This Application is a Divisional of Application No. 09/453,313, filed December 2, 1999; which is a Continuation-in-Part Application of U.S. Application No. 09/204,117, filed December 2, 1998 (now U.S. Patent 6,413,768), and also claims priority to U.S. Provisional Application No. 60/158,738, filed October 12, 1999; the above noted prior applications are all hereby incorporated by reference.

**At page 15, the paragraph encompassing lines 17-18 is amended as follows:**

Figure 3A-H: Flow cytometry histograms of GFP fluorescence for CVD 908-*htrA* carrying expression vectors with the *hok-sok* post-segregational killing system.

**At page 15, the paragraph encompassing line 19 is amended as follows:**

Figures 4A-DB: Complete pGEN2 nucleotide sequence (SEQ ID NO: 1), comprising nucleotides 1-41964199.

**At page 15, the paragraph encompassing line 20, is amended as follows:**

Figures ~~Figure~~ 5A-B: Partial pGEN3 nucleotide sequence (SEQ ID NO: 2), comprising nucleotides 1201-2397 ~~2400~~ and showing the sequence of *ori15A*.

**At page 15, the paragraph encompassing line 21 is amended as follows:**

Figures ~~Figure~~ 6A-C: Partial pGEN4 nucleotide sequence (SEQ ID NO: 3), comprising nucleotides 1201-38483850 and showing the sequence of *ori101*.

**At page 15, the paragraph encompassing lines 24-25 is amended as follows:**

Figures ~~Figure~~ 8A-C: Flow cytometry histograms of GFP fluorescence for expression plasmids pGEN91, pGEN111, pGEN121, pGEN193, and pGEN222.

**At page 23, the paragraph encompassing lines 16-18 is amended as follows:**

The basic structure of these vectors is represented in Figure 1, and the composite gene sequence for the vector ~~pGEN-2~~pGEN2 (SEQ ID NO: 1) is represented in Figure 4; Figures 5 & 6 show specific composite sequences for the origins of replication in pGEN3 and pGEN4 respectively.

**At page 43, the paragraph encompassing lines 7-22 is amended as follows:**

These data clearly show that when driving expression of *gfpuv* within the live vector strain CVD 908-*htrA*,  $P_{ompC1}$  and  $P_{ompC3}$  are inducible with increasing osmolarity, although the basal level of transcription is still noteworthy in both cases. The results observed under conditions of low osmolarity further support our observations using solid media that  $P_{ompC1}$  drives higher heterologous antigen expression than  $P_{ompC3}$ . Since  $P_{ompC3}$  was noted to possess the intended 3'-terminal *Bgl*III site, which was not detected for  $P_{ompC1}$ , we determined the nucleotide sequence for  $P_{ompC1}$  to perhaps detect point mutation(s) which might explain the strength of  $P_{ompC1}$ . The only differences identified were located at the 3'-terminus of the cassette. The intended sequence within this region was 5'...catataacAGATCTtaatcatccacAGGAGGatctgATG-3' (SEQ ID NO: 4) (from left to right, upper case denotes the *Bgl*III site, ribosome binding site, and GFPuv start codon respectively); the actual sequence proved to be 5'...catataacAGATC**GATCT**taa**A**catccacAGGAG**GtA**tctgATG-3 (SEQ ID NO: 5) (inserted or changed bases denoted with underlined bold upper case). These changes detected within the *ompC1* promoter sequence are apparently responsible for increasing the observed strength of  $P_{ompC1}$  by an unknown mechanism, since neither the basic *ompC* promoter sequence, nor the optimized ribosome binding site have been spontaneously altered.

**At page 50, lines 6-28 is amended as follows:**

**PRIMER 1:**

5'-gaattcGCGCGCTTCGCGATTCAGTCGCGTTCCTTCACA  
GCTGGCGCAGGGGCGATTACTGATGAA-3' (SEQ ID NO: 6)

**PRIMER 2:**

5'-cccgGAGTCTCCTGAATACGTTTCATAAAAGTGTA  
ACGCGTGAGTGTAACCATTTCCACGTAGC-3' (SEQ ID NO: 7)

**PRIMER 3:**

5'-cccggGTAAAAAACTCAAAGCGTTATTTGCATTTTCGC  
TATAGTTCTCGTCTGCTGAAATGCCTGGTGT-3' (SEQ ID NO: 8)

**PRIMER 4:**

5'-gaattcCATTTTCTATCAATAAATTACTATTAGTTTTGTCT  
TCTAACCAAGCCTCTATTTTATGAGTATCCTCTTCAG-3' (SEQ ID NO: 9)

**PRIMER 5:**

5'-gctagcATGGCCAGCAGAGGCGTAAACAAGGTGATTCT  
CGTTGGTAATCTGGGCCAGGACCCGGAAGTACGC-3' (SEQ ID NO: 10)

**PRIMER 6:**

5'-gctagcTCAGAACGGAATGTCGTCGTCAAATCCATTG  
GCGGTTCTGTTAGACGGCGCTGGCGCG-3' (SEQ ID NO: 11)

At pages 56, after line 6 to Page 58, line 5 (Table 9), is amended as follows:

TABLE 9					
Primer number	Sequence <sup>1</sup>	Cassette created	GenBank Accession Number	Region of Homology <sup>2</sup>	Region of Complementarity <sup>3</sup>
1	5'-GCAGGAAAGAACATGTGAG CCTAGGGCCAGCAAAAGGCCA GGAAC-3' (SEQ ID NO:12)	<i>oriE1</i>	J01749	2463-2507	
2	5'-CATGACCAAAATCCCTTA ACTAGTGTTTTAGATCTACT GAGCGTCAGAC CCCG-3' (SEQ ID NO: 13)	"	"		3197-3145
3	5'-CGGGGTCTGACGCTCAGT AGATCTAAAACACTAGTTAA GGGATTTTGGTCATG-3' (SEQ ID NO: 14)	<i>bla</i>	"	3145-3197	
4	5'-GCTGTCAAACATGAGAA TTCTAGAAGACGAAAGGGC CTCGTGATACGCC-3' (SEQ ID NO: 15)	"	"		17 - 1, 4361-4330
5	5'-ACAGCCTGCAGACAG ATCTTGACAGCTGGATCG CACTCTGGTATAATTGGG AAGCCCTGCAAAG -3' (SEQ ID NO: 16)	<i>aphA-2</i>	V00618	1-64	
6	5'-CGAAGCCCAACCTTTCAT AGAAGCTAGCGGTGGATCC GAAATCTCGTGATGGCAGGT TG-3' (SEQ ID NO: 17)	"	"		1044-986

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**A8702**

7	5'-AACAAAGCGTTATAGGAA TTCTGTGGTAGCA-3' (SEQ ID NO: 18)	<i>P<sub>ompC</sub></i>	K00541	4 - 33	
8	5'-ACTTTCATGTTATTAAGA TCTGTTATATG-3' (SEQ ID NO: 19)	"	"		498-469
9	5'-AGATCTTAATCATCCACAG GAGGCTTCTGATGAGTAAA GGAGAAGAACTTTTCAC TGG-3' (SEQ ID NO: 20)	<i>gfpuv</i>	U62636	289 - 317	
10	5'-GCTAGCTCATTATTTGT AGAGCTCATCCATGC-3' (SEQ ID NO: 21)	"	"		1008-983
11	5'-AGATCTGAATTCTAGAT CATGTTTGACAGCTTATCAT CGATAAGCTTTAATGCG-3' (SEQ ID NO: 22)	<i>tetA</i>	J01749	4 - 41	
12	5'-AGATCTTATCAGGTCGAG GTGGCCCGGCTCCATGCACC GCGACGCAACGCG-3' (SEQ ID NO: 23)	"	"		1275-1234
13	5'-CGCGAATTCTCGAGACAA ACTCCGGGAGGCAGCGTGAT GCGGCAACAATCACACGGAT TTC-3' (SEQ ID NO: 24)	<i>hok-sok- tetA</i>	X05813	2 - 48	
14	5'-ATGAGCGCATTGTTAGA TTTCATTTTTTTTTCTCCTT ATTTTCTAGACAACATCAGC AAGGAGAAAGG -3' (SEQ ID NO: 25)	"	J01749, X05813		108 - 86, 580 - 559
15	5'-CCTTTCTCCTTGCTGAT GTTGTCTAGAAAATAAGG AGGAAAAAAAAAATGAAAT CTAACAATGCGCTCAT-3' (SEQ ID NO: 26)	"	X05813, J01749	559 - 580, 86 - 108	
16	5'-GCTACATTTGAAGAGAT AAATTGCACTGGATCCTAG AAATATTTTATCTGATTAA TAAGATGATC-3' (SEQ ID NO: 27)	<i>ori15A</i>	X06403		1461-1397
17	5'-CGGAGATTTCTTGAA GATGCCTAGGAGATACTT AACAGGGAAGTGAGAG-3' (SEQ ID NO: 28)	"	"	780 - 829	
18	5'-GTCTGCCGATTGCTTA TCCTGGCGGATCCGGTTGA CAGTAAGACGGGTAAGCCT GTTGAT-3' (SEQ ID NO: 29)	<i>ori101</i>	X01654	4490-4550	

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19	5'- <u>CCTAGGTTTCACCTGTT</u> CTATTAGGTGTTACATGCTG TTCATCTGTTACATTGTCGAT CTG-3' (SEQ ID NO: 30)	"	"		6464-6408
20	5'-AGGCTTAAGTAGCACCC TCGCAAGATCTGGCAAATC GCTGAATATTCCTTTTGTG TCCGAC-3' (SEQ ID NO: 31)	<i>par</i>	X01654		4918-4858
21	5'-GAGGGCGCCCCAGCTGG CAATTCTAGACTCGAGCAC TTTTGTTACCCGCCAAACA AAACCCAAAAACAAC-3' (SEQ ID NO: 32)	<i>aphA2- parA</i>	V00618, X04268	38 - 16, 1 - 37	
22	5'-AGAAGAAAAATCGAATTC CAGCATGAAGAGTTTCAGAA AATGACAGAGCGTGAGCAA GTGC-3' (SEQ ID NO: 33)	"	X04268		1704-1644
23	5'-CGAAGCCCAACCTTTCA TAGAA <u>ACTAGT</u> GGTGGAA TCGAAATCTCGTGATGGCA GGTTG-3' (SEQ ID NO: 34)	"	V00618		1044 - 986
24	5'-GTTGTTTTTGGGTTTTGTT TGGCGGGTAACAAAAGTGC <u>TCGAGTCTAGAATTGCCAGC</u> TGGGGCGCCCTC-3' (SEQ ID NO: 35)	"	X04268, V00618	37 - 1, 16 - 38	